

CLAIMS:

1. A bivalent binding molecule comprising two or more binding domains to two or more epitopes of the same 7 transmembrane G protein-coupled receptor, wherein the binding domains are coupled to each other.
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2. The bivalent binding molecule of claim 1 wherein at least one binding domain is an aptamer.
- 10 3. The bivalent binding molecule of claim 2, wherein said aptamer is a SELEX-derived aptamer.
4. The bivalent binding molecule of claim 1, wherein all binding domains are aptamers.
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5. The bivalent binding molecule of claim 1, wherein one binding domain is an aptamer and the other binding domains are non-aptamer binding domains.
- 20 6. The bivalent binding molecule of claim 1 wherein the binding domains are coupled to each other via a linker.
7. The bivalent binding molecule of claim 6 wherein said linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyvinyl alcohol, hydrocarbons, polyacrylates and amino-, hydroxy-, thio or carboxy- functionalized silicones, proteins, peptides, polynucleotides, monosaccharides, oligosaccharides, cyclodextrins, dextran and liposomes.
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8. The bivalent binding molecule of claim 2 wherein the aptamer binding domain is coupled at the 3' end to another binding domain.
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9. The bivalent binding molecule of claim 2 wherein the aptamer binding domain is coupled at the 5' end to another binding domain.

10. The bivalent binding molecule of claim 2 wherein said aptamer is
5 modified at the 2', 5 or 8 position.

11. The bivalent binding molecule of claim 1, wherein said 7 transmembrane G protein-coupled receptor is selected from the receptors in Table 1.

10 12. A bivalent binding molecule to a 7 transmembrane G protein-coupled receptor, wherein said bivalent binding molecule comprises a first and second aptamer to a first and second epitope of the same 7 transmembrane G protein-coupled receptor, said bivalent binding molecule identified by the method comprising:

15 a) identifying said first aptamer to said first epitope by the method comprising:
i) preparing a first candidate mixture of nucleic acids;
ii) contacting said first candidate mixture of nucleic acid with said first epitope, wherein nucleic acids having an increased affinity to said first epitope may be partitioned from the remainder of the first candidate mixture;
20 iii) partitioning said increased affinity nucleic acids from the remainder of the first candidate mixture; and
iv) amplifying said increased affinity nucleic acids, whereby said first aptamer to said first epitope may be identified;

25 b) identifying said second aptamer to said second epitope by the method comprising:
i) preparing a second candidate mixture of nucleic acids;
ii) contacting said second candidate mixture of nucleic acid with said second epitope, wherein nucleic acids having an increased affinity to said second epitope may be partitioned from the remainder of the first candidate mixture;
30 iii) partitioning said increased affinity nucleic acids from the remainder of the first candidate mixture; and

- iv) amplifying said increased affinity nucleic acids, whereby said second aptamer to said second epitope may be identified; and
- c) coupling said first aptamer to said second aptamer, whereby said bivalent binding molecule may be identified.

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13. A bivalent binding molecule to a 7 transmembrane G protein-coupled receptor, wherein said bivalent binding molecule comprises a first and second aptamer to a first and second epitope of said same 7TM G protein-coupled receptor, wherein said bivalent binding molecule is identified by a method comprising:

10 a) preparing a bivalent binding molecule library generated according to a method comprising:

15 i) generating a first library of aptamers selected through the SELEX procedure for binding to said first epitope of said 7 transmembrane G protein-coupled receptor said aptamers having a 3' fixed sequence, and producing the double-stranded form of said first library of aptamers;

20 ii) generating a second library of aptamers selected through the SELEX procedure for binding to said second epitope of said 7 transmembrane G protein-coupled receptor, said aptamers having a 5' fixed sequence identical to the 3' fixed sequence of the aptamers of said first library, and producing the double-stranded form of said second library of aptamers;

25 iii) mixing said first and second libraries under conditions which promote interlibrary annealing;

iv) forming bivalent binding molecules by enzymatically extending the recessed 3' ends while copying the 5' extensions of each annealed pair, to yield a double-stranded bivalent binding molecule library;

v) amplifying said double-stranded bivalent binding molecule library;

30 b) contacting said bivalent binding molecule library with said 7 transmembrane G protein-coupled receptor, wherein bivalent binding molecules having an increased affinity to said first and second epitopes of said 7 transmembrane G protein-

coupled receptor may be partitioned from the remainder of the bivalent binding molecule library;

5 c) partitioning said increased affinity bivalent binding molecules from the remainder of said bivalent binding molecule library;

 d) amplifying said increased affinity bivalent binding molecules to yield a mixture increased affinity bivalent binding molecules having increased affinity to said first and second epitopes, whereby bivalent binding molecules to a 7 transmembrane G protein coupled receptor having affinity to a first and second epitope may be identified.

10 14. A bivalent binding molecule to a 7 transmembrane G protein-coupled receptor, wherein said bivalent binding molecule comprises an aptamer to a first epitope coupled to a non-aptamer binding domain which binds to a second epitope of the same receptor, wherein the bivalent binding molecule is identified according to a method comprising:

15 a) preparing a blended candidate mixture of bivalent binding molecules comprising a candidate mixture of nucleic acid sequences coupled to a non-aptamer binding domain which binds to said second epitope of the receptor;

20 b) contacting said 7 transmembrane G protein-coupled receptor with said blended candidate mixture of bivalent binding molecules, wherein bivalent binding molecules having an increased affinity to the 7 transmembrane G protein-coupled receptor relative to the blended candidate mixture may be partitioned from the remainder of the candidate mixture;

25 c) partitioning the increased affinity bivalent binding molecules from the remainder of the blended candidate mixture; and

 d) amplifying the increased affinity bivalent binding molecules to yield an enriched mixture of bivalent binding molecules, whereby bivalent binding molecules to a 7 transmembrane G protein-coupled receptor may be identified.

30 15. A bivalent binding molecule to a 7 transmembrane G protein-coupled receptor, wherein said bivalent binding molecule comprises an aptamer to a first epitope on a first extracellular domain of said receptor coupled to a non-aptamer binding domain

which binds to a second epitope on a second extracellular domain of the same 7TM G protein-coupled receptor, wherein said bivalent binding molecule is identified according to a method comprising:

- a) identifying an aptamer to said first epitope of said 7 transmembrane G protein-coupled receptor by the method comprising:
 - i) preparing a candidate mixture of nucleic acids;
 - ii) contacting said candidate mixture with said first epitope, wherein nucleic acids having an increased affinity to said first epitope relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;
 - iii) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
 - iv) amplifying the increased affinity nucleic acids to yield an enriched mixture of nucleic acids, whereby an aptamer to said first epitope of said 7 transmembrane G protein-coupled receptor may be identified; and
- b) coupling said aptamer to a non-aptamer binding domain which binds to said second epitope of said 7 transmembrane G protein coupled receptor to yield a bivalent binding molecule.

16. A bivalent binding molecule to a 7 transmembrane G protein-coupled receptor, wherein said bivalent binding molecule comprises an aptamer coupled to a second binding domain, said aptamer being an aptamer of non-natural handedness having binding affinity to the natural configuration of a first epitope, wherein said bivalent binding molecule is identified according to a method comprising:

- a) identifying said aptamer of non-natural handedness by the method comprising:
 - i) synthesizing a peptide enantiomer of the natural configuration of an amino acid sequence corresponding to a first epitope of said 7 transmembrane G protein-coupled receptor;
 - ii) contacting said peptide enantiomer with a candidate mixture of nucleic acids of natural handedness, wherein nucleic acids of natural handedness having

an increased affinity to the peptide enantiomer relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

iii) partitioning the increased affinity nucleic acids of natural handedness from the remainder of the candidate mixture;

5 iv) amplifying the increased affinity nucleic acids of natural handedness;

v) identifying the sequences of said increased affinity nucleic acids of natural handedness; and

10 vi) synthesizing the enantiomers of said increased affinity nucleic acids of natural handedness to yield a mixture of increase affinity nucleic acids of non-natural handedness, whereby an aptamer of non-natural handedness to the natural configuration of said first epitope may be identified; and

15 b) covalently coupling said aptamer of non-natural handedness to a second binding domain of said second epitope of said 7 transmembrane G protein-coupled receptor, whereby a bivalent binding molecule of said 7 transmembrane G protein-coupled receptor may be identified.